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Structural Features in the KshA Terminal Oxygenase Protein That Determine Substrate Preference of 3-Ketosteroid 9 alpha-Hydroxylase Enzymes

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1 **Supplemental Table 2.** Primers used in this study

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Chimer	Template	Primer name	Sequence (5'-3')
	<i>kshA1</i>	KshA1-c768g-F ^a	GGATACGACGTCGAGTCGGTCCTGATCAACTGCCACTACC
		KshA1-c768g-R ^a	GGTAGTGGCAGTTGATCAGGACCGACTCGACGTCGTATCC
	<i>kshA5</i>	KshA5-g612a-F ^a	AAGAACGTCTTCGAAGGCCACACCGCCACGCAG
		KshA5-g612a-R ^a	TGCGTGGCGGTGTGGCCTTCGAAGACGTTCTTG
	<i>kshA5</i>	KshA5-c786g-F ^a	CGAGACCATCCTGATCAACTGCCACTACCCGGTGAGC
		KshA5-c786g-R ^a	GCTCACCGGGTAGTGGCAGTTGATCAGGATGGTCTCG
<i>kshA1</i> _{A5loop} <i>kshA1</i> _{A5TG}	<i>kshA1</i>	pA1A5-13-F2	CGATCCGATGCAAGCTATTT
		pA1A5-13-R2 long	TCCTCACGACCGGTACCGCGCATGAACTGGCTGG
	<i>kshA1</i>	pA1A5-13-F2 long	AACGCCGAAGTGCATCCGATGCAAGCTATTT
		pA1A5-13-R2	ACCGCGCATGAACTGGCTGG
	<i>kshA5</i>	pA1A5-13-F1	ACCGGTCGTGAGGACGTCAT
		pA1A5-13-R1 long	TTGCATCGGATCGCAGTTCGGCGTTGGGGTCGTC
	<i>kshA5</i>	pA1A5-13-F1 long	AGTTCATGCGCGGTACCGGTCGTGAGGACGTCAT
		pA1A5-13-R1	CAGTTCGGCGTTGGGGTCGTC
<i>kshA5</i> _{A1loop}	<i>kshA5</i>	pA1A5-16-F2	CGTTCCGAGGCAACCTATTT
		pA1A5-16R2 long	CTCACGGGCCTGCGAGTGCATGTACTGCGTGG
	<i>kshA5</i>	pA1A5-16-F2 long	ATGATCGGAAGCCGTTCCGAGGCAACCTATTT
		pA1A5-16-R2	CGAGTGCATGTACTGCGTGG
	<i>kshA1</i>	pA1A5-16-F1	CAGGCCCGTGAGGACACCCGTCGG
		pA1A5-16-R1 long	TGCCTCGGAACGGCTTCCGATCATCTTCGGTTGA
	<i>kshA1</i>	pA1A5-16-F1 long	TACATGCACTCGCAGGCCCGTGAGGACACCC
		pA1A5-16-R1	GCTTCCGATCATCTTCGGTTGA
<i>kshA1</i> _{A5β201-210}	<i>kshA1</i>	pA1A5-19-F ^b	phos-ACCGCCACGCAGTACATGCACTCGCAGGCCCGTGAGGACA
		pA1A5-19-R	GTGTCTTTCGAAGACGTTCTTGAAGTACACCGGGAAGGAG
<i>kshA5</i> _{A1β207-216}	<i>kshA5</i>	pA1A5-22-F ^b	phos-GTCGCCAGCCAGTTCATGCGCGGTACCGGTCGTGAGGAC
		pA1A5-22-R	GTGGCCCTCGAAGACGTTCTTGAAGTAGCGCGGGAACGA
<i>kshA1</i> _{A5β230-238}	<i>kshA1</i>	pA1A5-25-F ^b	phos-GGCAACCTATTTTCGGTCCGTCGTACATGATCGACGATCTCG
		pA1A5-25-R	TCGGATCGGCTTCCGATCATCTTCGGTTGACCGTTCG
<i>kshA5</i> _{A1β236-244}	<i>kshA5</i>	pA1A5-28-F ^b	phos-TGCAAGCTATTTTCGGCCCGTCCTTCATGATCGACTGGCTC
		pA1A5-28-R	TCGGAACGCAGTTCGGCGTTGGGGTCGTCGTAGTTGGT
<i>kshA1</i> _{D242W}	<i>kshA1</i>	KshA1-D242W-F ^a	CCCGTCCTTCATGATCGACTGGCTCGTCTACGAGTACGAG
		KshA1-D242W-R ^a	CTCGTACTCGTAGACGAGCCAGTCGATCATGAAGGACGGG
<i>kshA5</i> _{W248D}	<i>kshA5</i>	KshA5-W248D-F ^a	TCCGTTCGTACATGATCGACGATCTCGAATCCGATGCCAAC
		KshA5-W248D-R ^a	GTTGGCATCGGATTCGAGATCGTCGATCATGTACGACGGA

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4 ^aMutated residues are underlined, ^bthe stretch of residues corresponding to *kshA5*
5 (pA1A5-19-F and pA1A5-25-F) or *kshA1* (pA1A5-22-F or pA1A5-28-F) is shown in
6 bold.

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